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L3: Entry 1 of 1 File: USPT Feb 27, 2001

DOCUMENT-IDENTIFIER: US 6194210 B1
TITLE: Hepatitis A virus culture process

US Patent No. (1):
6194210

Detailed Description Text (14):
Once the microcarriers have been equilibrated in the medium and temperature (30.degree.-37.degree. C.) of choice, cells, preferably in late log phase, are seeded into the culture vessel. For small scale work, NUNC CELL FACTORIES (NCFs), which are small multilamellar cell culture units within which cells may be grown in monolayer according to the manufacturer's directions, are convenient for this purpose. The inoculum cell concentration is 5-10 cells per microcarrier bead which corresponds to about 100,000 (1.times.10.sup.5) cells/mL at the bead loadings used. For a small microcarrier culture of about 600 mL, about 6.times.10.sup.7 cells are required. From one confluent ten-layer NCF, approximately 5.times.10.sup.8 cells may be harvested using trypsin. These ratios are easily scaled upward for larger microcarrier culture inocula by using additional NCF or, if necessary, a seed microcarrier culture. Once the cells have been trypsin harvested and neutralized with serum containing media, they are pelleted by low-speed centrifugation and resuspended in medium containing about a 10% iron supplemented calf serum, or they are simply diluted with media containing about 10% iron supplemented calf serum. By using one part of cell inoculum to nine parts of microcarrier/serum free culture medium, a 1% iron supplemented calf serum final concentration is attained. This ratio may naturally be modified by modifying the concentration of serum added to the resuspended cell inoculum or by modifying the inoculum to bioreactor volume ratios. A 1% serum concentration at pH 7.6-7.9 was found to provide uniform cell attachment.

Detailed Description Text (50):
In this experiment, the cell density at plant was 1.5.times.10.sup.5 cells/ml which corresponds to 8600 cells/cm.sup.2 MC. By day 2 small aggregates of 2-5 MCs formed which grew to 10-30 MCs by day 5. Subsequently, some of the 10-30 MC aggregates combined to form .about.50 MC aggregates. It is quite clear that cell growth occurs through the aggregation process. No single MCs were present in the culture and cell growth within the void space between the MCs was apparent. The cell growth profile, illustrated in FIG. 1 indicates the cultures reached a cell density of 2-3 MM cells/ml on day 5. Nuclei counts on day 6 were lower since the larger aggregates became lodged in a constriction in the sample line. Since the particle concentration decreases about 50 fold due to aggregation, the culture changes from rather opaque with the single MCs to quite translucent. Thus, monitoring the progress of the culture on-line with a turbidity probe is possible. No floating cells due to cell sloughing were observed in effluent samples during the 28 day run. The nuclei counts from the bioreactor harvest at the end of the run also indicate that the culture remained stable throughout the infection period. We have found that the aggregates were very stable once formed, even under extremes in pH, presence of EDTA, and higher agitation rates. Since the aggregates readily breakup in the presence of trypsin, the aggregates are most likely stabilized by the creation of a extracellular matrix composed of collagens secreted by the cells.

Detailed Description Text (89):
Solohill glass coated microcarriers at 40 g/liter, 60 g/liter and 80 g/liter were

prepared in a microcarrier spinner system as outlined in example 2 and inoculated with MCR-5 cells expansded in NUNC CELL FACTORIES in a similar manner as outlined in example 3. The cells were perfused at target rates of 1, 1.5, and 2 volumes per day and with the increasing rate corresponding to the higher bead loading. The perfusion operation was similar to example 4. Cells were grown for 4 to 5 days, at which time they were infected with working seed at approximately an infection ratio of 1 harvested working seed cell to 15 target cells in the microcarrier culture. The cell density at the time of infection varied from 2 to 4 million cells/ml. The working seed was prepared by infecting a ten layer NUNC CELL FACTORY with stockseed at an MOI of 1:125 and harvested with trypsin after 48 hours of infection. A sample of infected microcarrier culture was harvested at 6 hour time points starting at 30 hours by decanting off the media with four washes of phosphate buffered saline followed by resuspension in a stabilizer formation. The aggregates were broken up by fluid shear using a syringe needle. At approximately 48 hours post infection the entire volume of each of the three spinners was harvested using the fluid shear methodology described in example 6. The aggregates were more difficult to break up without the presence of detergent as described in example 6. Therefore, more fluid shear by increasing the linear velocity through the orifices is required.

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L25: Entry 1 of 1 File: USPT Aug 31, 1993

DOCUMENT-IDENTIFIER: US 5241053 A
TITLE: Fused proteins comprising glycoprotein gD of HSV-1 and LTB

US Patent No. (1):
5241053

Detailed Description Text (4):
The antigens used for vaccines include antigens of viruses whose hosts are animals, such as antigens of herpesviruses including herpes simplex virus (HSV), varicella-zoster virus (VZV) and cytomegalovirus (CMV); antigens of retroviurses including human immunodeficiency virus (HIV) and adult T cell leukemia virus (HTLV-I); antigens of hepadonaviruses including hepatitis B virus (HBV); antigens of togaviruses including non-A, non-B hepatitis virus (HCV and HEV) and Japanese encephalitis virus; antigens of picornaviruses including hepatitis A virus (HAV); antigens of orthomyxoviruses including influenza virus; antigens of parvoviruses, antigens of papovaviruses; antigens of adenoviruses; antigens of poxviruses; antigens of reoviruses; antigens of paramyxoviruses; antigens of rhabdoviruses; antigens of arenaviruses; and antigens of coronaviruses; antigens of pathogenic protozoa such as a malarial antigen; and antigens of pathogenic protozoa such as a malarial antigen; and antigens of pathogenic bacteria such as a Bordetella pertussis antigen.